Osteoarthritis and Cartilage 18 (2010) 1355–1357

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Brief Report

Cathepsin K inhibition reduces CTXII levels and joint pain in the guinea pig model of spontaneous osteoarthritis

J.J. McDougall †*, N. Schuelert †, J. Bowyer ‡

† *Department of Physiology & Pharmacology, University of Calgary, 3330 Hospital Drive NW, Calgary, AB T2N 4N1, Canada*
‡ *Respiratory & Inflammation Research Area, AstraZeneca, Charnwood, Loughborough, Leicestershire LE11 5RH, UK*

ARTICLE INFO

Article history:
Received 18 May 2010
Accepted 30 July 2010

Keywords:
Animal models
Biomarkers
Joint pain
Osteoarthritis
Proteinases
Electrophysiology

SUMMARY

Cathepsin K is a cysteine proteinase which is believed to contribute to osteoarthritis (OA) pathogenesis. This brief report evaluates the effect of the novel selective cathepsin K inhibitor AZ12606133 on cartilage metabolism in the Dunkin–Hartley guinea pig model of spontaneous OA. In parallel, electrophysiological studies were performed to determine whether acute and chronic treatment with the cathepsin K inhibitor could alter joint nociception. Acute treatment of OA knees with AZ12606133 had no effect on joint afferent nerve activity; however, prolonged (1 month) administration of the cathepsin K inhibitor delivered *via* a chronically implanted osmotic pump significantly reduced mechanosensitivity in response to both non-noxious and noxious joint movements. Urinal concentrations of the cartilage breakdown products cross-linked C-telopeptides of type II collagen (CTXII) were also reduced by chronic cathepsin K inhibition. These data suggest that prolonged AZ12606133 administration can reduce cartilage turnover and joint nociception in the Dunkin–Hartley guinea pig model of spontaneous OA.

Experiments were carried out on male 9-month-old Dunkin–Hartley guinea pigs (0.78–1.17 kg). Animals of this age typically show moderate signs of osteoarthritis (OA) and joint pain[1,2]. Animals were housed in individual cages at room temperature with a 12 h light: 12 dark cycle and free access to food and water. All animal handling and surgical procedures carried out received prior approval by the University of Calgary Animal Care Committee.

Electrophysiological recording of knee joint afferents

A more detailed overview of the joint afferent recording technique has been previously reported[3]. Briefly, animals were deeply anaesthetised by ethyl carbamate (25% Urethane, 2 g/kg i.p.) and a medial incision was made in the right hindlimb of the guinea pig. The saphenous nerve was then isolated in the inguinal region and cut centrally to avoid spinal reflexes. Thin fascicles were dissected from the main trunk of the nerve and placed over a platinum recording electrode. The receptive field of the recorded nerve fibre was determined by gently palpating the surface of the hindlimb with a thin glass rod. Only afferents with a discrete receptive field within the joint were used for experimental assessment. Single unit action potentials were amplified and all the signals underwent analogue-to-digital conversion by a data acquisition system (CED1401, Cambridge Electronic Design, Cambridge, UK).

The muscle relaxant gallamine triethiodide (50 mg/kg i.v.) was injected to eliminate neural interference arising from hindlimb muscle afferents. The right femur was clamped in a stereotaxic frame and the right hindpaw secured in a rigid Perspex boot thereby immobilising the hip and ankle respectively. Rotational movements could then be applied to the knee joint and resultant torque levels were standardised and measured by a force transducer and torque meter (MVD2510; Hottinger-Baldwin Messtechnik, Darmstadt, Germany). In the acute pain studies, the ipsilateral saphenous artery was cannulated at a point distal to the medial articular artery to allow local close intra-arterial injection of test agents. Joint afferent recordings were carried out for 1 min prior to knee movement to determine the level of spontaneous nerve activity (if any). The knee joint was then hyper-rotated and held in this position for 10 s. This noxious movement was then repeated at 1, 3, 5, 7, 9, 12 and 15 min following close intra-arterial administration of either vehicle or AZ12606133 (10 μg or 100 μg in 100 μl bolus). The number of action potentials evoked during each movement was ascertained.

In chronic pain experiments, AZ12606133 was administered continuously for 28 days to OA guinea pigs *via* an implanted mini osmotic pump (Alzet Osmotic Pumps, Cupertino, CA, USA). Animals were deeply anaesthetised (2–5% isoflurane in 100% O_2; 1 L/min)

* Address correspondence and reprint requests to: Jason J. McDougall, Department of Physiology & Pharmacology, University of Calgary, 3330 Hospital Drive NW, Calgary, AB T2N 4N1, Canada. Tel: 1-403-2204507; Fax:1-403-2103949.
E-mail address: mcdougaj@ucalgary.ca (J.J. McDougall).

1063-4584/$ – see front matter
doi:10.1016/j.joca.2010.07.014

and under aseptic conditions a mini osmotic pump (Alzet 2ML4, 2.5 μl/h; total volume = 2 ml) containing either vehicle or AZ12606133-007 (0.5 μg/kg/day) was placed subcutaneously in the dorsum of the guinea pig. Animals were allowed to recover before being prepared for electrophysiological recording. In these experiments, afferent nerve activity was recorded in response to both normal joint rotation (40 mNm) and hyper-rotation (65 mNm). Each movement lasted 10 s and was repeated three times. The mean firing rate of the nerve fibre over the three movements was calculated as before.

Chronic administration of AZ12606133 and cartilage degradation

On the final day of chronic administration of AZ12606133, (Day 28), urine was collected over a 6 h period by placing guinea pigs in metabolism cages. The urine was stored at −20°C until later analysed for evidence of cartilage turnover using the cross-linked C-telopeptides of type II (CTXII) collagen assay, (Cartilaps ELISA, Nordic Bioscience, Herlev, Denmark). Samples were normalised to creatinine levels to correct for urine volume differences.

Pharmacokinetic analyses were carried out on day 26 of AZ12606133-dosed animals. Blood samples were taken *via* cardiac heart puncture from terminally anaesthetised animals and aliquotted into lithium–heparin coated tubes. Samples were centrifuged at 12,000*g* for 3 min and the supernatant (serum) was frozen at −20°C until analysed for drug concentration.

Statistics

All data were normally distributed and expressed as means and 95% confidence intervals for "*n*" observations. The effect of acute administered drugs between animal groups was analysed by either one- or two-way analysis of variance (ANOVA). Urinary CTXII levels and effect of chronic administered drugs were analysed using a Student's *t* test, (two-tailed). $P < 0.05$ was considered statistically significant.

Effect of AZ12606133 on joint nociception

Acute local administration of AZ12606133 (10 μg or 100 μg close intra-arterial) had no observable effect on joint afferent spontaneous activity nor on movement-evoked firing rate (Fig. 1). A two-factor ANOVA confirmed that there was no significant difference between animals given vehicle and those receiving the cathepsin K inhibitor ($P = 0.29$; $n = 6$–11).

Knee joint afferents in old guinea pigs often fire even at rest. This spontaneous activity in animals having received a slow infusion of vehicle for a month was found to be 52.9 (lower limit: 24.27, upper limit: 81.53) action potentials/min ($n = 26$ fibres). In contrast, the cohort of age-matched guinea pigs treated with AZ12606133 exhibited a firing rate of only 8.8 (lower limit: 0.33, upper limit: 17.21) action potentials/min ($n = 18$ fibres). This reduction in spontaneous activity between the two groups was statistically significant ($P = 0.035$, unpaired Student's *t* test). Moreover, chronic administration of AZ12606133 caused both non-noxious and noxious movement-evoked afferent firing rate to fall by approximately 50% (Table I).

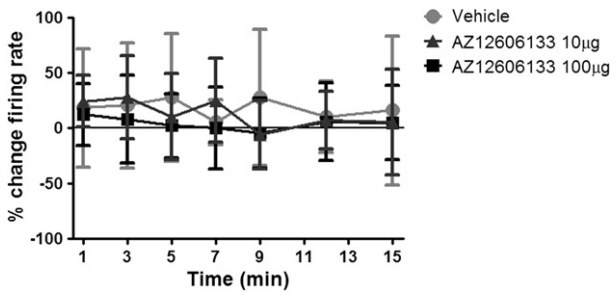

Fig. 1. Effect of acutely administered AZ12606133 on knee joint afferent firing rate in response to hyper-rotation of the knee. Blockade of cathepsin K had no effect on OA joint mechanosensitivity during this time period. Data are means and 95% confidence intervals. $n = 6$–11 fibres.

Effect of AZ12606133 on cartilage metabolism

Chronic dosing of guinea pigs with AZ12606133 at 0.5 μg/kg/day gave a mean blood concentration of 8.8 (lower limit: 6.128, upper limit: 11.47) ng/ml. Vehicle treated animals had urine CTXII levels of 1.123 (lower limit: 0.658, upper limit: 1.588) ng/ml (per mmol/L creatinine) ($n = 8$). In contrast, CTXII levels from AZ12606133-treated animals were lower by approximately 63% to 0.405 (lower limit: 0.218, upper limit: 0.598) ng/ml (per mmol/L creatinine, $P = 0.0045$, unpaired Student's *t* test; $n = 8$).

This brief report aimed to elucidate the effect of a novel selective cathepsin K inhibitor (AZ12606133) on cartilage integrity and joint nociception using the Dunkin–Hartley guinea pig model of spontaneous OA. Cathepsin K is a cysteine proteinase which is believed to contribute to OA pathogenesis since it is upregulated in OA joints near areas of cartilage destruction and bone resorption[4,5]. Here we used CTXII in the urine as a biomarker of cartilage metabolism since this molecule is a product of type II collagen turnover. In the 9-month-old guinea pig, the growth plate of many joints is not yet ossified so urinary CTXII may reflect turnover of mineralized articular cartilage[6] as well as growth plate cartilage in this model system. Chronic treatment of Dunkin–Hartley guinea pigs for 1 month with AZ12606133 caused a 63% reduction in CTXII excretion thereby demonstrating the inhibition of cartilage (growth plate and/or mineralized articular cartilage) turnover with cathepsin K inhibition. One of the major limitations of this study was that effects of cathepsin K inhibition on cartilage were not able to be evaluated by histopathology or structural imaging. An improvement in joint morphology by AZ12606133 requires validation in a larger more in depth study. Another limitation relates to the lack of measurement of CTXII in the synovial fluid. This could have provided stronger evidence for a direct effect of the cathepsin K inhibitor on articular cartilage that could have been particularly helpful in the absence of histology or imaging.

The effect of AZ12606133 on joint pain was also assessed in the guinea pig OA model by electrophysiologically recording from knee joint mechanonociceptive nerves. Acute, local administration of AZ12606133 had no observable effect on joint afferent spontaneous activity nor on movement-evoked afferent firing rate. This absence of neuromodulation suggests that AZ12606133 has no direct effect on joint nociceptors which is not unexpected as there is no evidence directly linking cathepsin K with neural function. In contrast, chronic administration of AZ12606133 over a 1 month period significantly reduced joint afferent firing frequency in

Table I
Response of joint mechanosensory nerves to non-noxious and noxious rotation following chronic administration of either vehicle or AZ12606133. The cathepsin K inhibitor significantly reduced afferent firing rate with both types of movement. Data are means and 95% confidence intervals (lower limit, upper limit). Differences in firing rate were analysed using an unpaired Student's *t* test

		Firing rate/movement	*N*	*P*-value
Non-noxious	Vehicle	30.85 (20.25, 41.45)	24	
	AZ12606133	15.68 (7.46, 23.90)	16	(0.037)
Noxious	Vehicle	67.01 (42.71, 91.31)	24	
	AZ12606133	34.44 (22.15, 46.73)	16	(0.038)

response to both non-noxious and noxious mechanical stimuli. This observation suggests that AZ12606133-induced anti-nociception is likely related to the potential impact of cathepsin K inhibition on disease activity. An improvement in joint integrity would lead to enhanced joint stability and consequently a reduction in stimulation of joint mechanosensitive nerves[7]. Furthermore, since one of the primary origins of OA pain is thought to be activation of exposed subchondral nociceptors either directly or *via* cartilage degradation products[7,8], a recovery in articular cartilage integrity could reduce neural activation within this region. An alternative explanation for the reduction in joint nociception following chronic AZ12606133 administration could be that long term cathepsin K inhibition leads to a gradual change in nerve function culminating in a loss in joint mechanosensitivity. In a time-resolved fluorescence resonance energy transfer assay, AZ12606133 was found to be a potent inhibitor of human (0.99 nM) and guinea pig (0.88 nM) full length recombinant cathepsin K. AZ12606133 also has at least 1000 fold selectivity over human cathepsin L, S and B (the family members that display highest homology to cathepsin K), when tested in similar assays. Whilst off target pharmacology cannot be completely excluded, the potency and selectivity of AZ12606133 suggest that the effects on joint nociception observed during chronic administration are attributable to selective inhibition of cathepsin K and not other cathepsins.

The physiological relationship between structural changes in the OA joint and the onset and duration of pain is complex and poorly understood, but the concept that modifying or protecting the joint from deterioration will subsequently reduce or prevent the onset of pain is worthy of investigation in pre-clinical models. One of the drawbacks to this type of work is the availability of appropriate, validated animal models in which this relationship is robust and amenable to testing of novel agents. Rodent models such as intra-articular monoiodoacetate or joint injury have been shown to be useful models for testing analgesics[9–11], but are considered less than ideal for replicating the structural pathology of human OA. Conversely, the Dunkin–Hartley guinea pig model of naturally-occurring OA is more attractive from the standpoint of clinical relevance since there is no artificial derangement of the joint and the pathology is similar to human disease[1]. Crucially, however, there is a poor correlation between disease severity and nociception in this model[2] and guinea pigs are less amenable to standard pain behavioural tests, such as altered weight bearing, as both knees are equally diseased. In the absence of suitable surrogate measures of joint pain in the guinea pig, joint nociception has been objectively measured in these animals by recording electrophysiologically from knee joint primary afferents in response to non-noxious and noxious movements of the knee[2,12]. In addition, whilst there are no proven drugs which alter disease progression clinically, the utility of this animal model in identifying disease modifying OA drugs is evident by the findings that doxycycline, which lessens joint space narrowing in man[13], partially protects guinea pigs from cartilage loss[14]. These independent findings suggest that the guinea pig model is ideal for exploring structural modification and joint nociception.

In summary, this preliminary study found that prolonged infusion of AZ12606133 to OA guinea pigs reduced CTXII excretion and joint mechanonociception. Based on these data, further studies are warranted to evaluate the potential analgesic and chondroprotective effects of this cathepsin K inhibitor.

Author contributions

Conception & design of experiments: JJMcD, NS, JB.
Data acquisition/assembly/analysis/interpretation: JJMcD, NS, JB.
Writing and critical revision of article: JJMcD, NS, JB.
Approval of final submitted version of article: JJMcD, NS, JB.

Conflict of interest

This work was supported by a contract provided by AstraZeneca, UK. JB has shares in AstraZeneca, UK.

Acknowledgements

The authors acknowledge salary support from the Alberta Heritage Foundation for Medical Research and the Arthritis Society of Canada.

References

1. Bendele AM, Hulman JF. Spontaneous cartilage degeneration in guinea pigs. Arthritis Rheum 1988;31:561–5.
2. McDougall JJ, Andruski B, Schuelert N, Hallgrimsson B, Matyas JR. Unravelling the relationship between age, nociception and joint destruction in naturally occurring osteoarthritis of Dunkin Hartley guinea pigs. Pain 2009; 141:222–32.
3. McDougall JJ, Hanesch U, Pawlak M, Schmidt RF. Participation of NK1 receptors in nociceptin-induced modulation of rat knee joint mechanosensitivity. Exp Brain Res 2001;137:249–53.
4. Morko J, Kiviranta R, Mulari MT, Ivaska KK, Vaananen HK, Vuorio E, *et al*. Overexpression of cathepsin K accelerates the resorption cycle and osteoblast differentiation in vitro. Bone 2009;44:717–28.
5. Morko JP, Soderstrom M, Saamanen AM, Salminen HJ, Vuorio EI. Up regulation of cathepsin K expression in articular chondrocytes in a transgenic mouse model for osteoarthritis. Ann Rheum Dis 2004;63:649–55.
6. Garnero P, Piperno M, Gineyts E, Christgau S, Delmas PD, Vignon E. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. Ann Rheum Dis 2001;60:619–26.
7. McDougall JJ. Arthritis and Pain. Neurogenic origin of joint pain. Arthritis Res Ther 2006;8:220–9.
8. Niv D, Gofeld M, Devor M. Causes of pain in degenerative bone and joint disease: a lesson from vertebroplasty. Pain 2003;105: 387–92.
9. Bove SE, Calcaterra SL, Brooker RM, Huber CM, Guzman RE, Juneau PL, *et al*. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. Osteoarthritis Cartilage 2003;11:821–30.
10. Bove SE, Laemont KD, Brooker RM, Osborn MN, Sanchez BM, Guzman RE, *et al*. Surgically induced osteoarthritis in the rat results in the development of both osteoarthritis-like joint pain and secondary hyperalgesia. Osteoarthritis Cartilage 2006; 14:1041–8.
11. McDougall JJ, Watkins L, Li Z. Vasoactive intestinal peptide (VIP) is a modulator of joint pain in a rat model of osteoarthritis. Pain 2006;123:98–105.
12. McDougall JJ. Pain and OA. J Musculoskelet Neuronal Interact 2006;6:385–6.
13. Brandt KD, Mazzuca SA, Katz BP, Lane KA, Buckwalter KA, Yocum DE, *et al*. Effects of doxycycline on progression of osteoarthritis: results of a randomized, placebo-controlled, double-blind trial. Arthritis Rheum 2005;52:2015–25.
14. Bowyer J, Heapy CG, Flannelly JK, Waterton JC, Maciewicz RA. Evaluation of a magnetic resonance biomarker of osteoarthritis disease progression: doxycycline slows tibial cartilage loss in the Dunkin Hartley guinea pig. Int J Exp Pathol 2009; 90:174–81.